

# National Health and Nutrition Examination Survey 2003-2004

---

## Documentation, Codebook, and Frequencies

### MEC Laboratory Component: Erythrocyte Protoporphyrin

**Survey Years:**  
**2003 to 2004**

**SAS Export File:**  
**L39\_C.XPT**



January 2006

# NHANES 2003–2004 Data Documentation

## Laboratory Assessment: Lab 39 - Erythrocyte Protoporphyrin

Years of Coverage: 2003–2004

First Published: January 2006

Last Revised: N/A

---

### Component Description

#### Protoporphyrin

The objectives of this component are: 1) to provide data for monitoring secular trends in measures of nutritional status in the U.S. population; 2) to evaluate the effect of people's habits and behaviors such as physical activity and the use of alcohol, tobacco, and dietary supplements on people's nutritional status; and 3) to evaluate the effect of changes in nutrition and public health policies including welfare reform legislation, food fortification policy, and child nutrition programs on the nutritional status of the U.S. population.

These data will be used to estimate deficiencies and toxicities of specific nutrients in the population and subgroups, to provide population reference data, and to estimate the contribution of diet, supplements, and other factors to serum levels of nutrients. Data will be used for research to further define nutrient requirements as well as optimal levels for disease prevention and health promotion.

### Eligible Sample

**Protoporphyrin:** Participants of both genders aged 3–5 years and females aged 12–49 years were tested.

### Description of Laboratory Methodology

#### Protoporphyrin:

Porphyrins and heme components are extracted from whole blood into a 4:1 mixture of ethyl acetate-acetic acid. Porphyrins are then separated from heme by back-extraction into a 1.5 M hydrochloric acid solution and quantitatively determined by molecular fluorometry using a spectrofluorometer calibrated with protoporphyrin IX (PPIX) standard solutions; however, the exact concentration of the standards must first be established using molecular absorbance, Beer's Law, and the millimolar absorptivity of PPIX.

The analytical method for EP routinely employed by the EP Lab is based largely on those originally described by Sassa *et al.* (1973) and Chisolm and Brown (1975). New York State's extraction method owes much to contributions from other public health labs, including CDC, and

closely follows the key elements of the consensus method for EP as published by the National Committee for Clinical Laboratory Standards (NCCLS C42-A\*, 2001). At the invitation of Dr. Sassa, the EP Laboratory's routine method for EP was published as Unit 8.8 in Current Protocols in Toxicology, 1999 by J. Wiley & Sons, Inc. Elements of this protocol are reproduced below, but a reprint of the original publication is available from the EP lab director.

### **Protoporphyrin**

The Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention performed testing from 1999 to 2001. The State of New York Department of Health, Wadsworth Center, Trace Elements Laboratory began testing from 2002 to 2004.

A detailed description of the laboratory method used can be found on the NHANES website.

### **Laboratory Quality Control and Monitoring**

The NHANES quality assurance and quality control (QA/QC) protocols meet the 1988 Clinical Laboratory Improvement Act mandates. A detailed quality control and quality assurance instruction was discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed QA/QC protocols.

A detailed description of the quality assurance and quality control procedures can be found on the NHANES website.

### **Data Processing and Editing**

Specimens were processed, stored and shipped to the State of New York Department of Health, Wadsworth Center, Trace Metals Laboratory, Albany, New York. Detailed specimen collection and processing instructions was discussed in the NHANES LPM. Read the LABDOC file for detailed data processing and editing protocols. The analytical methods were described in the **Description of the Laboratory Methodology** section.

There was no top coding in this file. See the lab39 Freqs link to determine "below detectable limit fill values" for this data.

### **Protoporphyrin**

The age range for protoporphyrin changed to males and females 3–5 years and females 12–49 years in 2003–2004 from 1 year and older

from the previous 2 years.

One derived variable was created in this data file. The formula for their derivation is as follows:

LBDEPPSI:

The protoporphyrin in  $\mu\text{g/dL}$  RBC (LBXEPP) was converted to  $\mu\text{mol/L}$  (LBDEPPSI) by multiplying by 0.0177.

Detailed instructions on specimen collection and processing can be found on the NHANES website.

## **Analytic Notes**

The analysis of NHANES 2003–2004 laboratory data must be conducted with the key survey design and basic demographic variables. The NHANES 2003–2004 Household Questionnaire Data Files contain demographic data, health indicators, and other related information collected during household interviews. They also contain all survey design variables and sample weights for these age groups. The phlebotomy file includes auxiliary information such as the conditions precluding venipuncture. The household questionnaire and phlebotomy files may be linked to the laboratory data file using the unique survey participant identifier SEQN.

## **References**

1. Sassa S, Granick JL, Granick S, Kappas A, Levere RD. Microanalyses of erythrocyte protoporphyrin levels by spectrophotometry in the detection of chronic lead intoxication in the subclinical range. *Biochem Med.* 1973;8:135–148.
2. Committee on Specifications and Criteria for Biochemical Compounds, National Research Council. Specifications and criteria for biochemical compounds, 3rd ed. Washington, D.C.: National Academy of Sciences, 1972.
3. Culbreth P, Walter G, Carter R, Burtis C. Separation of protoporphyrin and related compounds by reversed-phase liquid chromatography. *Clin Chem.* 1979; 25(6):605–610.
4. Gunter EW, Turner WE, Neese JW, Bayse DD. Laboratory procedures used by the Clinical Chemistry Division, Centers for Disease Control, for the Second Health and Nutrition Examination Survey (NHANES 1999+ II) 1976-80. Atlanta: Centers for Disease Control, 1981: 8-12.
5. Gunter EW, Miller DT. Laboratory procedures used by the Division

of Environmental Health Laboratory Sciences, Center for Environmental Health, Centers for Disease Control, for the Hispanic Health and Nutrition Examination Survey (HHANES) 1982-84. Atlanta: Centers for Disease Control, 1986: 9–13.

6. Gunter EW, Turner WE, Huff DL. Investigation of protoporphyrin IX standard materials used in acid-extraction methods, and a proposed correction for the millimolar absorptivity of protoporphyrin IX. *Clin Chem.* 1989;35(8):1601–1609.
7. Washington, D.C.: U.S. Government Printing Office, 1982.
8. Gunter EW, Lewis BL, Koncikowski SM. Laboratory methods used for the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. Centers for Disease Control and Prevention, Hyattsville, MD., 1996. VII-A-1 to VII-A-XII.
9. State of New York Department of Health, Wadsworth Center, Trace Metals Laboratory: Blumberg, W.E., Eisinger, J., Lamola, A.A., and Zuckerman, D.M. 1977a. The hematofluorometer. *Clin Chem.* 23:270–274.
10. Blumberg, W.E., Eisinger, J., Lamola, A.A., and Zuckerman, D.M. 1977b. Zinc protoporphyrin level in blood determined by a portable hematofluorometer: a screening device for lead poisoning. *J Lab Clin Med.* 89:712–723.
11. Bowers, M.A., Aicher, L.D., Davis, H.A., and Woods, J.S. 1992. Quantitative determination of porphyrins in rat and human urine and evaluation of urinary porphyrin profiles during mercury and lead exposures. *J Lab Clin Med.* 120:272–281.
12. Centers for Disease Control, 1975. Increased lead absorption and lead poisoning in young children [Report]. U.S. Department of Health, Education, and Welfare, Atlanta, GA.
13. Centers for Disease Control, 1978. Preventing lead poisoning in young children [Report]. U.S. Department of Health, Education, and Welfare, Atlanta, GA.
14. Centers for Disease Control, 1985. Preventing lead poisoning in young children [Report]. U.S. Department of Health and Human Services, Atlanta, GA.
15. Centers for Disease Control, 1991. Preventing lead poisoning in young children [Report]. U.S. Department of Health and Human Services, Atlanta, GA.
16. Chiba, M. and Sassa, S. 1982. Analysis of porphyrin carboxylic acids in biological fluids by high- performance liquid chromatography. *Anal Biochem.* 124:279–285.

17. Chisolm, J., Jr. and Brown, D.H. 1975. Micro-scale photofluorometric determination of "free erythrocyte pophyrin" (protoporphyrin IX). Clin Chem. 21:1669–1682.
18. Doran, D. and Mitchell, D.G. 1984. Problems in the determination of erythrocyte protoporphyrin by ethyl acetate-acetic acid extraction. Ann Clin Biochem. 21:141–145.
19. Gunter, E.W., Turner, W.E., and Huff, D.L. 1989. Investigation of protoporphyrin IX standard materials used in acid- extraction methods, and a proposed correction for the millimolar absorptivity of protoporphyrin IX. Clin Chem. 35:1601–1608.
20. Ho, J., Guthrie, R., and Tieckelmann, H. 1987. Quantitative determination of porphyrins, their precursors and zinc protoporphyrin in whole blood and dried blood by high-performance liquid chromatography with fluorimetric detection. J Chromatogr. 417:269–276.
21. Kammholz, L.P., Thatcher, L.G., Blodgett, F.M., and Good, T.A. 1972. Rapid protoporphyrin quantitation for detection of lead poisoning. Pediatrics. 50:625–631.
22. National Committee for Clinical Laboratory Standards., 1996. Erythrocyte protoporphyrin testing; Approved guideline. 1 pp. NCCLS Document C42-A, Wayne, PA.
23. Parsons, P.J., Reilly, A.A., and Hussain, A 1991. Observational Study of Erythrocyte Protoporphyrin as a Screening Test for Detecting Lead Exposure in Children: Impact of Lowering the Blood Lead Action Threshold Clin Chem. 37:216-225.
24. Piomelli, S. 1973. A micromethod for free erythrocyte porphyrins: the FEP test. J Lab Clin Med. 81:932-940.
25. Sagen, E. and Romslo, I. 1985. Determination of porphyrins by high performance liquid chromatography: fluorescence detection compared to absorbance detection. Scand. J Clin Lab Invest. 45:309-314.
26. Sassa, S., Granick, J.L., Granick, S., Kappas, A., and Levere, R.D. 1973. Studies in lead poisoning. I. Microanalysis of erythrocyte protoporphyrin levels by spectrophotometry in the detection of chronic lead intoxication in the subclinical range. Biochem Med. 8:135-148.
27. Sato, H., Ido, K., and Kimura, K. 1994. Simultaneous separation and quantification of free and metal-chelated protoporphyrins in blood by three-dimensional HPLC. Clin Chem. 40:1239-1244.

28. Schwartz, S., Berg, M.H., Bossenmaier, I., and Dinsmore, H. 1960. Determination of porphyrins in biological materials. In *Methods of biochemical analysis*, Vol VIII, D. Glick ed.) pp. 221-294. Interscience Publishers, Inc., New York.
29. Scoble, H.A., McKeag, M., Brown, P.R., and Kavarnos, G.J. 1981. The rapid determination of erythrocyte porphyrins using reversed-phase high performance liquid chromatography. *Clin Chim Acta*. 113:253-265.

## Locator Fields

**Title:** Erythrocyte Protoporphyrin

**Contact Number:** 1-866-441-NCHS

**Years of Content:** 2003–2004

**First Published:** January 2006

**Revised:** N/A

**Access Constraints:** None

**Use Constraints:** None

**Geographic Coverage:** National

**Subject:** Erythrocyte Protoporphyrin

**Record Source:** NHANES 2003–2004

**Survey Methodology:** NHANES 2003–2004 is a stratified multistage probability sample of the civilian non-institutionalized population of the U.S.

**Medium:** NHANES Web site; SAS transport files



**National Health and Nutrition Examination Survey  
Codebook for Data Production (2003-2004)**

**Erythrocyte Protoporphyrin (L39\_C)  
Person Level Data**

January 2006



<b>SEQN</b>	<b>Target</b>
	B(3 Yrs. to 5 Yrs.) F(12 Yrs. to 49 Yrs.)
<b>Hard Edits</b>	<b>SAS Label</b>
	Respondent sequence number
<b>English Text:</b> Respondent sequence number	
<b>English Instructions:</b>	

LBXEPP	Target		
	B(3 Yrs. to 5 Yrs.) F(12 Yrs. to 49 Yrs.)		
Hard Edits	SAS Label		
	Protoporphyrin(ug/dL RBC)		
English Text: Protoporphyrin(ug/dL RBC)			
English Instructions:			
Code or Value	Description	Count	Skip to Item
12 to 744	Range of Values	2665	
.	Missing	2572	

LBDEPPSI	Target		
	B(3 Yrs. to 5 Yrs.) F(12 Yrs. to 49 Yrs.)		
Hard Edits	SAS Label		
	Protoporphyrin(umol/L RBC)		
English Text: Protoporphyrin(umol/L RBC)			
English Instructions:			
Code or Value	Description	Count	Skip to Item
0.21 to 13.24	Range of Values	2665	
.	Missing	2572	